

Karyotypic variation in the long-whiskered catfish *Pimelodus blochii* Valenciennes, 1840 (Siluriformes, Pimelodidae) from the lower Tapajós, Amazonas and Trombetas Rivers

Ivanny Coelho da Fonseca^{1,4}, Luan Aércio Melo Maciel^{2,4},
Frank Raynner Vasconcelos Ribeiro³, Luís Reginaldo Ribeiro Rodrigues^{1,2,4}

1 Graduate Program in Amazonian Natural Resources – PPGRNA, Federal University of Western Pará – UFOPA, Av. Mendonça Furtado, 2ºAndar, Nº 2440, Bairro Aparecida CEP: 68040-050, Santarém, PA, Brazil **2** Graduate Program in BioSciences – PPGBio, Federal University of Western Pará – UFOPA, Av. Mendonça Furtado, 2ºAndar, Nº 2440, Bairro Aparecida CEP: 68040-050, Santarém, PA, Brazil **3** Water Science and Technology Institute – ICTA, Federal University of Western Pará, Campus Amazonia, Av. Mendonça Furtado, 2ºAndar, Nº 2946, Bairro Fátima CEP: 68040-050, Santarém, PA, Brazil **4** Genetics and Biodiversity Laboratory – LGBio, Federal University of Western Pará – UFOPA, Rua Vera Paz, s/n, Salé, 68035-110, Santarém, PA, Brazil

Corresponding author: Luís Reginaldo Ribeiro Rodrigues (luisreginaldo.ufpa@hotmail.com)

Academic editor: G. Furgala-Selezniov | Received 28 November 2017 | Accepted 28 June 2018 | Published 2 August 2018

<http://zoobank.org/E7737895-7F06-47EA-8C8A-7CF7D6BF491D>

Citation: Fonseca IC, Maciel LAM, Ribeiro FRV, Rodrigues LRR (2018) Karyotypic variation in the long-whiskered catfish *Pimelodus blochii* Valenciennes, 1840 (Siluriformes, Pimelodidae) from the lower Tapajós, Amazonas and Trombetas Rivers. Comparative Cytogenetics 12(3): 285–298. <https://doi.org/10.3897/CompCytogen.v12i3.22590>

Abstract

The genus *Pimelodus* LaCépède, 1803 comprises 35 formally recognized species distributed along the major neotropical river basins. Despite conservatism in diploid number with $2n=56$, an intense variation of chromosomal morphology (karyotypic formula) has been documented in *Pimelodus* species. In the present study, we analyzed karyotypes of 20 specimens, identified as *Pimelodus blochii* Valenciennes, 1840 and collected from the lower courses of the Tapajós, Amazonas and Trombetas Rivers. The karyotypes were characterized by Giemsa conventional staining, C-banding, silver staining (Ag-NOR) and fluorescent in situ hybridization (FISH) with 5S and 18S rDNA probes. The karyotypes showed $2n=56$ chromosomes in fish from the Tapajós River. In contrast, fish from the Amazonas and Trombetas Rivers had $2n=58$.

The nucleolus organizing regions were labeled on the short arm of an acrocentric chromosome as demonstrated by silver staining and FISH. Signals for 18S and 5S rDNA were co-localized on one chromosome pair. Our results demonstrate karyotypic divergence between Tapajós and Amazonas-Trombetas populations of *P. blochii*, interpreted as supporting the existence of a species complex in this taxon.

Keywords

Amazon basin, catfish, *Pimelodus*, rDNA, species complex

Introduction

The genus *Pimelodus* LaCépède, 1803 (Siluriformes, Pimelodidae) comprises 35 valid species exclusively distributed in neotropical freshwater drainages. It is commonly recorded in the Amazonas, Orinoco, Araguaia-Tocantins, São Francisco, and Paraná-Paraguay River basins. In the Amazon basin, seven species of *Pimelodus* have been recorded: *Pimelodus albofasciatus* Mees, 1974, *Pimelodus blochii* Valenciennes, 1840, *Pimelodus altissimus* Eigenmann & Pearson, 1942, *Pimelodus jivaro* Eigenmann & Pearson, 1942, *Pimelodus ornatus* Kner, 1858, *Pimelodus pictus* Steidachner, 1876, and *Pimelodus tetramerus* Ribeiro & Lucena, 2006 (Ferraris 2007, Eschmeyer and Fong 2017).

Cytogenetic analysis of 32 Pimelodidae family members revealed a conservative karyotypic macrostructure with $2n=56$ chromosomes save for a few exceptional karyotypes ($2n=50$) in *Calophysus macropterus* Lichtenstein, 1819, *Pinirampus pirinampu* Spix & Agassiz, 1829 and *Luciopimelodus plati* Valenciennes, 1835 species (Ramirez-Gil et al. 1998; Sánchez et al. 2000; Vasconcelos and Santos 2000). Diploid chromosome number variations were also reported in *Pimelodus fur* Lütken, 1874 and *Megalonema platanum* Günther, 1880 species samples with $2n=54$ and in *Pimelodus blochii* (Della-Rosa et al. 1980) species samples with $2n=58$ (Sánchez et al. 2000; Garcia and Moreira-Filho 2008; Carvalho et al. 2011).

Diploid chromosome numbers of eleven previously investigated *Pimelodus* species showed variation from 54 to 58 with eleven distinct karyotype formula (see Table 2 in the Discussion section). Additionally, karyotype variation with B chromosomes had been reported in *P. ortmanni* and *Pimelodus* sp. (Borin and Santos 2004).

So far, two distinct karyotypes were reported for two *Pimelodus blochii* populations; $2n=56$ for the Araguaia River, and $2n=58$ for the Amazon River (Swarça et al. 2007). Although the chromosome numbers of *P. blochii* were declared in three meetings and published in abstracts by Swarça et al. (2007), no karyotype image of the species is available in any peer-reviewed literature. Therefore, the taxon has been considered as poorly described in the scientific literature.

Rocha (2006) suggested that the name *P. blochii* is arbitrarily assigned to many different long-whiskered catfish in the Brazilian part of the Amazon Basin. Based on morphometric and molecular data, a large collection of *P. blochii* specimens was examined by Rocha (2006). It was demonstrated that the Brazilian specimens are distinct from *P. blochii* topotypes from Suriname and possibly represent a species complex with six undescribed taxa.

In the present paper, we investigate the karyotype of *Pimelodus blochii* from the lower portions of the Tapajós, Amazonas and Trombetas Rivers in order to evaluate their chromosomal features and contribute the debate on the species taxonomy. The karyotypes were characterized by conventional Giemsa staining, C-banding, silver staining (Ag-NOR) and fluorescent in situ hybridization (FISH) technique with 5S and 18S rDNA probes.

Material and methods

Samples and collection sites

Twenty (20) specimens were collected from four localities in the Tapajós, Amazonas and Trombetas Rivers (Table 1). The fish were captured by local fishermen using hooks and gillnets. The specimens were transferred to plastic tanks (50 L capacity) filled with water from the collection site and aerated with an aquarium pump. After cytogenetic procedures, the specimens were photographed, fixed in 10% formalin for 48 h, washed with running water and preserved with 70% ethanol. The voucher specimens were deposited in the Fish Collection of the Water Science and Technology Institute at Federal University of Western Pará, Brazil. External morphology and coloration features are shown in Fig. 1. The experimental procedures were approved by the Ethical Committee of Animal Research at Federal University of Western Pará (CEUA/UFOPA) under Protocol N. 10001/2015.

Chromosome preparation

Intra-abdominal colchicine (0.0125%) injection was performed at 0.01 ml/g (Bertollo et al. 1978) to stop cell division. The exposed fish were placed in an aerated tank for 40 min and euthanized with water that contains a lethal concentration of clove oil. The posterior kidney tissue was removed and minced in 6 ml of hypotonic KCl solution (0.075 M, 5.6 g/L). The cell suspension was incubated at 37 °C for 20 min and then fixed with fresh methanol-acetic acid (3:1 v/v) solution; the fixative was changed three times.

Table 1. Samples and collection sites of *Pimelodus blochii* in the Amazon Basin.

River	Collection sites	GPS Coordinates (datum WGS84)	n
Tapajós	Itaituba	4°16'12.6"S, 55°58'37.1"W	6
Amazonas	Santarém	2°25'8.0"S, 54°44'28.6"W	6
	Chicaia River, Almeirim	1°38'15.6"S, 52°57'46.2"W	4
Trombetas	Oriximiná	1°45'52.2"S, 55°52'18.8"W	4

Specimens: PO-22, PO-25, PO-27, PO-28, ITB-11, ITB14-18, STXVI-1, STXVI-2, STXVI-9, ALC-1-4, PML-5-7.

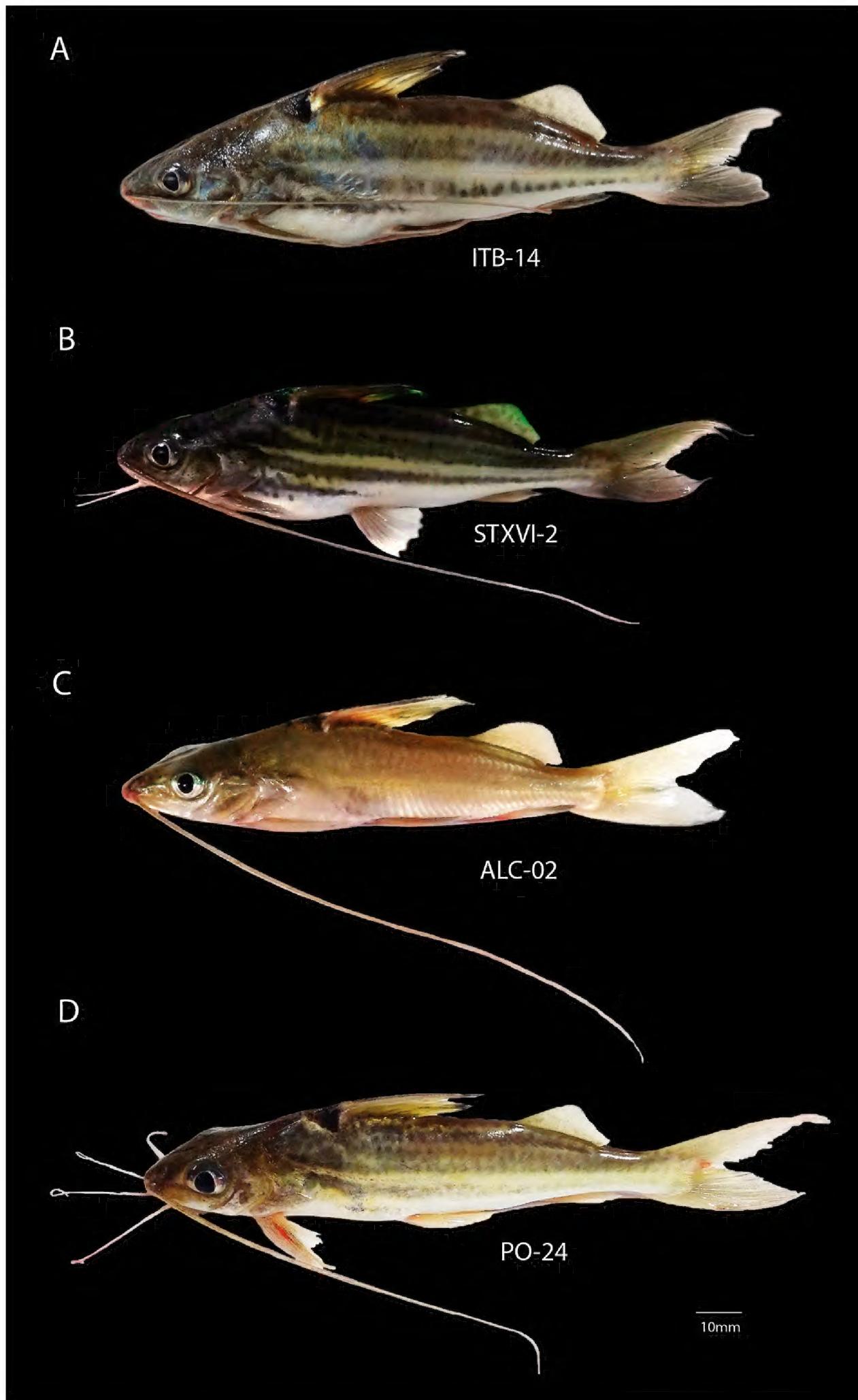


Figure 1. External morphology and coloration of *Pimelodus blochii* specimens examined in the present study. **A** specimen from the Tapajós River (ITB-14, SL=150 mm; W=55 g) **B** specimen from the lower Amazonas River at Santarém (STXVI-2, SL=145 mm; W=52 g) **C** specimen from the lower Amazonas River at Almeirim (ALC-2, SL=110 mm; W=9 g) **D** specimen from the Trombetas River (PO-22, SL=108 mm, W=33 g).

Chromosome staining, banding and FISH

Conventional staining was performed with 5% Giemsa solution (phosphate buffer, pH 6.8). The C-banding protocol from Sumner (1972) was followed with minor changes. The NORs were stained with silver nitrate following the Howell and Black (1980) technique.

FISH was used for mapping 18S and 5S rDNA loci (Pinkel et al. 1986). Double FISH experiments were processed with probes generated with 18Sf (5' CCG CTT TGG TGA CTC TTG AT 3') and 18Sr (5' CCG AGG ACC TCA CTA AAC CA 3') primers, as well as 5Sa (5-TAC GCC CGA TCT CGT CCG ATC) and 5Sb (5-CAG GCT GGT ATG GCC GTA AGC-3) PCR primers (Martins and Galetti 1999; Martins and Vicari 2012).

The PCR products were labeled by nick translation with biotin-14-dATP (BioNick Labeling System kit, Invitrogen/ThermoScientific, Waltham, Massachusetts, USA) and digoxigenin-11-dUTP (DIG-nick translation mix, Roche, Basel, Switzerland). The slides were treated with RNase solution (5 µl RNase 10 mg/mL diluted in 975 µl 2×SSC) for a short period of time. The fixed chromosomes were denatured in 70% formamide (pH 7.0 2×SSC) and heated at 70 °C for 5 min. The hybridization solution mixture was prepared with 20 µl formamide + 8 µl of 50% dextran sulfate + 4 µl of each probe + 4 µl of 20×SSC. The slides were incubated in 2×SSC solution in a humidified and heated (37 °C) chamber overnight.

Post-hybridization washes were performed with 15% formamide at 42 °C for 10 min, three washes in 0.1×SSC at 60 °C for 5 min, and 0.5% Tween20 at room temperature for 5 min. For signal detection, slides were placed in NFDM buffer (20 ml of 20×SSC, pH 7.0 + 5 g of powdered skim milk + 80 ml of distilled water) for 15 min, followed by two washes in 5% Tween20 for 5 min at room temperature.

The hybridized probes were applied in a mixture containing 20 µl anti-digoxigenin-rhodamine (1:200) (Roche, Basel, Switzerland) + 4 µl FITC-Avidin (1:100) (Sigma, St. Louis, Missouri, USA) + 26 µl of C buffer (0.1 M sodium bicarbonate, 0.15 M sodium chloride; pH 7.0) for 60 min. The slides were coated with anti-fading reagent Vectashield H-1000 (Vector Laboratories, Burlingame, California, USA) and chromosomes were counterstained with DAPI (1,2-diamidin-phenyl-indol).

Microscopy and karyotype analysis

At least 30 metaphases were counted to determine the diploid chromosome number. The best spread metaphase plates were photographed with a CCD camera (Moticam 10 MP) coupled to a Zeiss Axioskop40 microscope for conventional/banding images, and a Nikon Eclipse CI for FISH images. The contrast and brightness were adjusted with ADOBE PHOTOSHOP CS3. The chromosomes were arranged as metacentric (m), submetacentric (sm), subtelocentric (st) and acrocentric (t) following Levan et al. (1964).

Results

The diploid chromosome number was observed as 56 in the Tapajós River fish (Itaituba population). On the other hand, 2n=58 chromosomes were recorded from Amazonas (Santarém and Almeirim populations) and Trombetas Rivers (Oriximiná population) samples, with minor variation in the karyotypic formula, as 30m/sm+28a and 26m/sm+32a, respectively (Fig. 2).

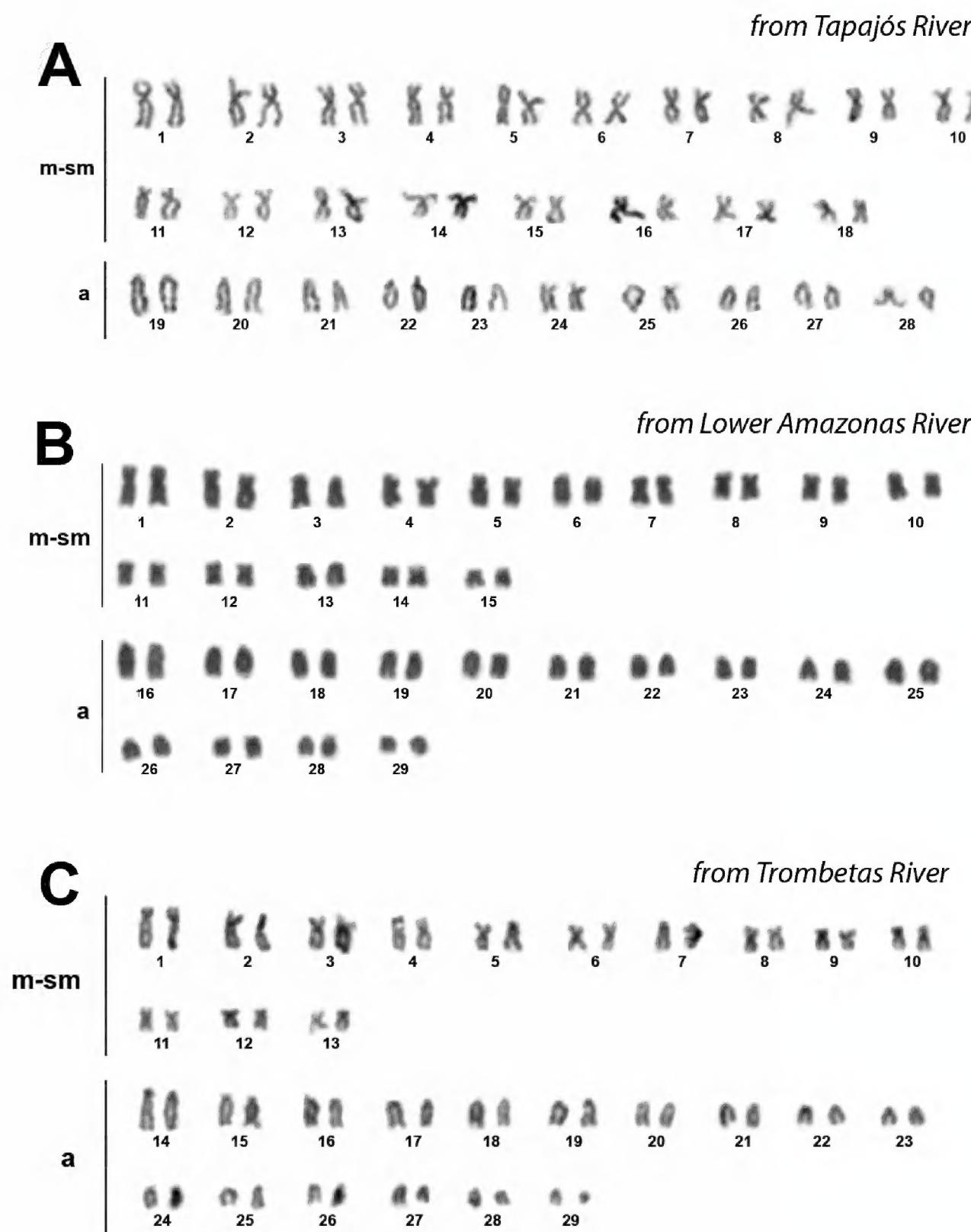


Figure 2. Giemsa-stained karyotypes of *Pimelodus blochii* from the Amazon Basin. **A** 2n=56 chromosomes (36m/sm + 20a) **B** 2n=58 chromosomes (30m/sm + 28a) **C** 2n=58 chromosomes (26m/sm + 32a). Scale bar: 10 μ m.

The C-banding results showed small amounts of constitutive heterochromatin in the centromeres. Terminal C-bands were observed in 18 to 24 chromosome pairs from the Tapajós River specimens (Fig. 3). A single NOR-bearing chromosome pair was detected by silver staining in all samples and labeled as the centromeric position (Fig. 4a–d). These sites were compatible with the 18S rDNA locus as demonstrated by FISH (Fig. 4e–h).

The 5S rDNA probe showed distinct localizations among the samples. Co-localization of 5S and 18S rDNA to a single chromosome pair was detected in the Trombetas and Amazonas Rivers populations (Almeirim population); this syntenic pattern also occurred in the Santarém population but on just one homologous chromosome. The Tapajós River specimens' karyotypes showed a distinct position for 18S and 5S rDNA (Figs 4, 5).

Discussion

The karyotype macrostructure of *Pimelodus blochii* from the Tapajós, Amazonas and Trombetas Rivers are compatible with a previous report (Swarça et al. 2007) (Table 2). The diploid chromosome number was found to be 58 (30m/sm+28a) in the lower Amazonas population; this is compatible with the Solimões River population (Della-Rosa et al. 1980). The specimens from the Trombetas River conserved 2n=58 but shifted the

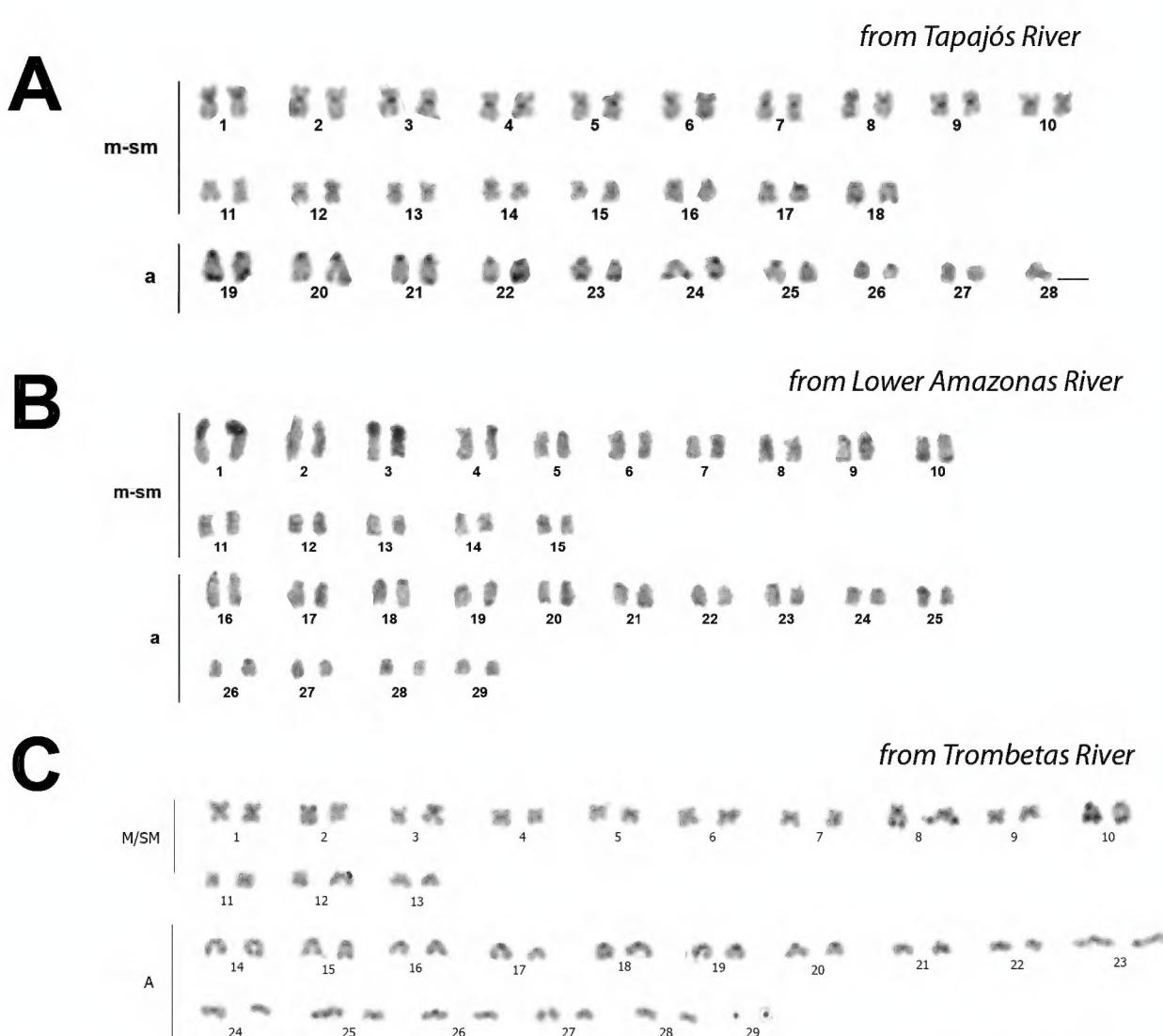


Figure 3. C-banded karyotypes of *Pimelodus blochii* from the Amazon River basin. Scale bar: 10 μ m.

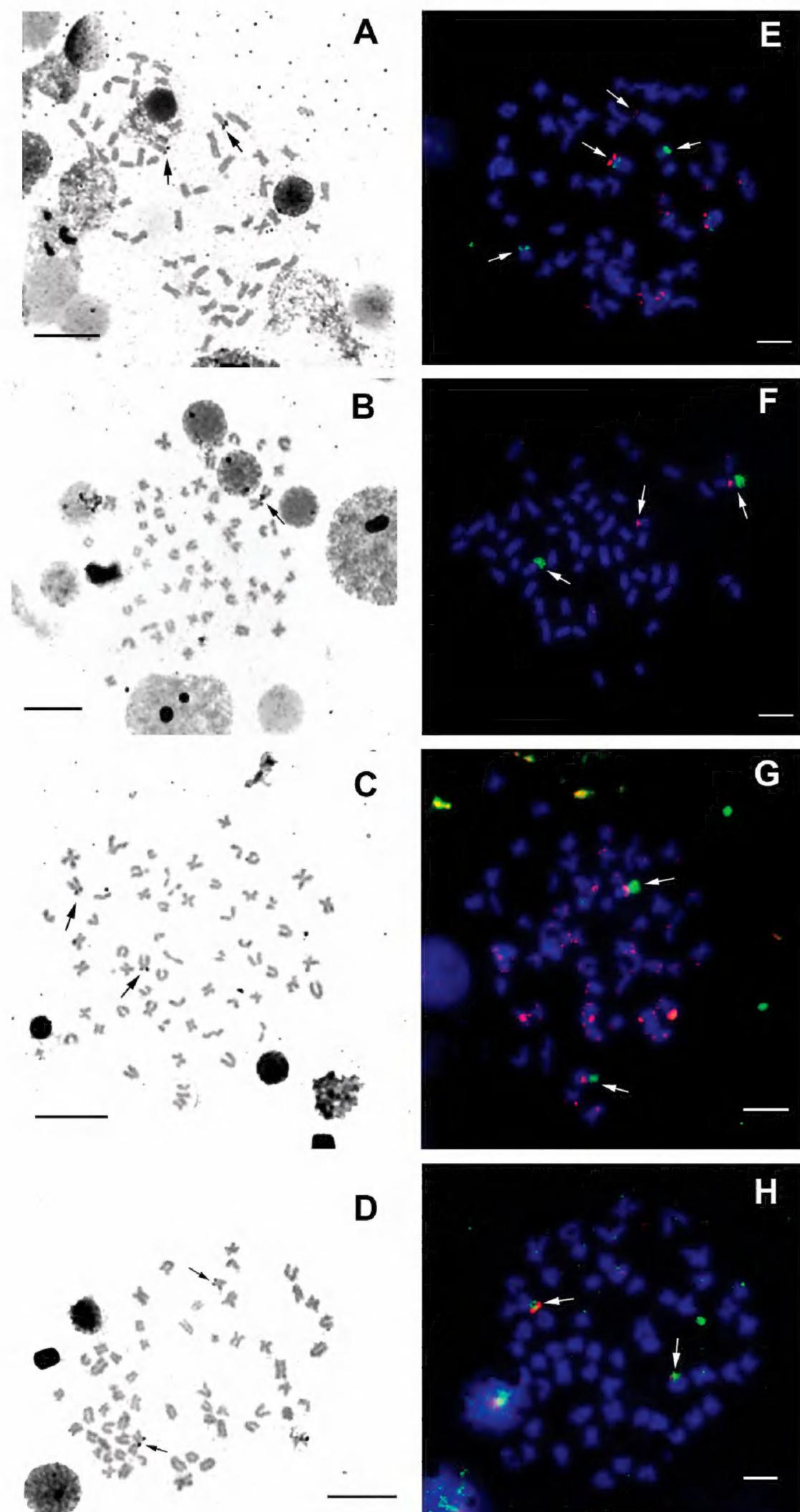


Figure 4. Metaphases of *Pimelodus blochii* showing NOR by silver staining (left) and double FISH of 18S rDNA (green) and 5S rDNA (red) (right). Specimens from the Tapajós River (**A, E**); from the Lower Amazonas River at Santarém (**B, F**); at Almeirim (**D, H**); and from the Trombetas River (**C, G**). Scale bar: 10 µm.

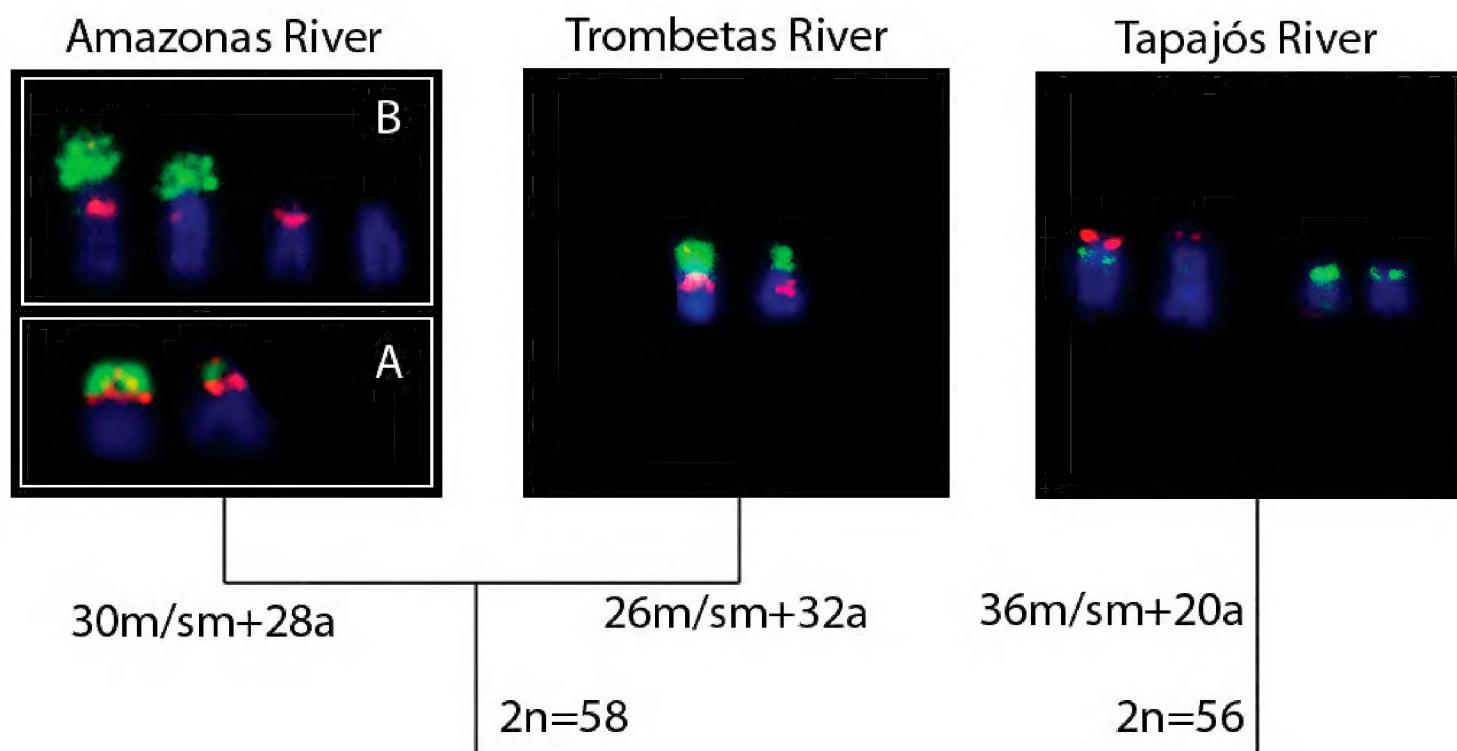


Figure 5. Variation of 18S rDNA (green signal) and 5S rDNA (red signal) chromosomal sites among *Pimelodus blochii* populations from Amazon basin. The samples from Amazonas River showed two distinct patterns: one observed in the Almeirim population (**A**) and other observed in the Santarém population (**B**).

karyotypic formula to 26m/sm+32a; this could be a result of pericentric inversions. On the other hand, *Pimelodus blochii* from the Tapajós River with $2n=56$ (36m/sm+20a) showed a resemblance to the population from the Araguaia River (Barra do Garça, Mato Grosso State) (Farias et al. 2000).

Despite extensive conservatism in diploid number, variation in karyotypic formula has been frequently detected (Swarça et al. 2007). Pericentric inversions can explain such modifications of chromosomal morphology without alteration of the diploid number and have been previously demonstrated, such as in *Pimelodus maculatus* and *Pimelodus* sp. (Dias and Foresti 1993).

A single NOR (one pair) is the most common pattern observed in the *Pimelodus* karyotypes (Swarça et al. 2007). This pattern was confirmed for *P. blochii* in the present study. The Ag-NOR sites were coincident with the rDNA 18S FISH signal and showed co-localization with the 5S, a rare condition previously observed in *Pimelodus britskii* (Neto et al. 2011). In the Order Siluriformes, the synteny of 18S and 5S rDNA cistrons has been reported for *Pimelodus* (Neto et al. 2011; present study), *Imparfinis* Eigenmann et Norris 1900 (Ferreira et al. 2014), *Ancistrus* Kner 1854 (Favarato et al. 2016), *Hemibagrus* Bleeker 1862 (Supiwong et al. 2014), *Corydoras* LaCepède 1803 (Rocha et al. 2016), *Panaqolus* Isbrücker et Schraml 2001 (Ayres-Alves et al. 2017) and *Bunocephalus* Kner 1855 (Ferreira et al. 2017). This syntenic arrangement is interpreted as being less adaptive since in eukaryotes the 45S rRNA genes are transcribed by RNA polymerase I, whereas the 5S are transcribed by RNA polymerase III. This means both processes occur in separate nuclear territories (Amarasinghe and Carlson 1998). Additionally, a linked configuration of 18S and 5S rDNA arrays could favor an undesired disruption of both tandem repeats by means of unequal crossing-over (Martins and Wasko 2004).

Table 2. Compiled data from the literature on karyotypic traits in *Pimelodus* species. m=metacentric, sm=submetacentric, st=subtelocentric, a=acrocentric, q=long arm, t=terminal, c=centromeric, inter=interstitial, peri=pericentromeric.

Species	2n	Karyotypic formula	18S	5S	References
<i>Pimelodus fur</i>	54	32m+8sm+6st+8a	q, t, sm	q inter m, q peri sm	5
<i>P. microstoma</i>	56	22m+22sm+6st+6a	q, t, st	peri sm, q peri st	1; 2
<i>P. argenteus</i>	56	24m+16sm+12st+4a			3
<i>P. britskii</i>	56	24m+18sm+8st+6a	q, t, st	p inter sm, q t st	4
<i>P. maculatus</i>	56	32m+12sm+12st	q, t, sm	q inter m, q t sm q peri sm	5; 6
<i>P. absconditus</i>	56	24m+18sm+8st+6a	—	—	7
<i>P. mysteriosus</i>	56	26m+20sm+2st+8a	—	—	3; 8
<i>P. ornatus</i>	56	18m+22sm+6st+10a	—	—	9; 7
<i>P. ortmanni</i>	56	24m+18sm+8st+6a	—	—	10; 11; 12
<i>P. paranaensis</i>	56	22m+22sm+4st+8a	—	—	13
<i>P. blochii</i>	56/58	36m/sm+20st/a; 30m/sm+28a; 26m/sm+32a	q, c, a	q, c, a	14; 15; 16; 17

References: 1) Fenocchio et al. (1994); 2) Souza et al. (2004); 3) Souza et al. (2003); 4) Neto et al. (2011); 5) Garcia and Filho (2008); 6) Mazzuchelli et al. (2007); 7) Borin and Santos (2002); 8) Girardi (2015); 9) Abucarma and Santos (1996); 10) Borin and Santos (2004); 11) Terencio et al. (2001); 12) Margarido and Gavasso (2000); 13) Treco and Dias (2009); 14) Della-Rosa et al. (1980); 15) Farias et al. (2000); 16) Silva et al. (2004); 17) (present study).

Eigenmann (1912) recognized varieties (A and B) of *P. blochii* from Guyana. The A variety has an ashy body pigmentation without dots or stripes, whereas the B variety exhibits four lateral body stripes with the fourth stripe possibly absent or fragmented into dots. *Pimelodus albofasciatus* (Mees 1974) maintains a close resemblance to the B variety but is distinguished based on eye morphology and dorsal spine length (Ribeiro and Lucena 2006).

Our specimens collected from the Chicaia River, a tributary of the lower Amazonas (Almeirim population), have the typical pigmentation for the A variety, whereas the specimens from the Santarém population, collected at the confluence of the Amazonas and Tapajós Rivers, had the B variety pigmentation. Although both populations conserved the diploid number 2n=58 and karyotypic formula (30m/sm+28a), they diverged in their 18S and 5S rDNA locations (Fig. 5). The specimens from the Trombetas River also have the B variety pigmentation, but diverge in the karyotypic formula (26m/sm+32a) and 18S and 5S rDNA locations (Fig. 5). The most differentiated karyotype was observed in specimens from the Tapajós population; these had a clearly distinct diploid number, karyotypic formula and 18S and 5S location. In general, their coloration resembles the B variety, but their karyotypic distinctiveness leads us to suggest that these specimens may be a new, undescribed *Pimelodus* species.

Ribeiro and Lucena (2006), Azpelicueta et al. (2008), and Lucinda et al. (2016) discussed three distinct patterns of pigmentation among species of *Pimelodus*. According to Lucinda et al. (2016), among the species formally described, a striped-pattern is shared by *P. albicans*, *P. albofasciatus*, and *P. tetramerus*. However, our results, as well

as those of Ribeiro and Lucena (2006), suggest that the diversity of *Pimelodus* with blackish stripes along its flanks that inhabit the waters of the Amazonas River basin is greater than the diversity currently described. This includes undescribed species that have commonly been misidentified as *P. blochii*. *P. blochii* from the lower Amazonas River occurs as two morphotypes (A and B) distinguished through body pigmentation and characterized by a $2n=58$ karyotype with minor variations. Additional studies of this group are needed in order to clarify the evolutionary dynamics of the 18S and 5S rRNA genes as well as to acquire morphological and molecular data to evaluate the taxonomy and phylogeny of *Pimelodus* species.

Conclusions

The populations of *Pimelodus blochii* from the lower courses of Amazonas, Tapajós and Trombetas rivers presented differentiated karyotypes based on variation in diploid number and chromosome morphology. The specimens collected from the Tapajós River, with $2n=56$, are clearly distinguished from the others and may constitute a new, undescribed *Pimelodus* species.

Acknowledgments

This research was supported by grants from CAPES/PRO-AMAZÔNIA (Auxpe-3318/2013). The authors are grateful to the native fishermen (Haroldo Leite, Mr Naldo, Mr Nelson, William Vasconcelos, Janison Vasconcelos, Mr Claudenido) for helping us with fieldwork and to the LGBIO/UFOPA staff for help in the laboratory. ICF received Mastership from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This work is part of the Project “Estudos Citogenéticos e Citogenômicos da Biodiversidade da Amazônia, com implementação de avanços técnicos” (Cytogenetic and cytogenomic studies of Amazonian biodiversity implementing technical advances) coordinated by Dra Cleusa Y. Nagamachi (UFPA).

References

Abucarma M, Santos ICM (1996) Caracterização cromossômica de duas espécies da família Pimelodidae (Pisces, Siluriformes). Proceedings of VI Simpósio de Citogenética Evolutiva Aplicada a Peixes Neotropicais, São Carlos, October 9–11, 1996, Brazil, 73.

Amarasinghe V, Carlson JE (1998) Physical mapping and characterization of 5S rRNA genes in douglas-fir. *Journal of Heredity* 89: 495–500. <https://doi.org/10.1093/jhered/89.6.495>

Ayres-Alves T, Cardoso AL, Nagamachi CY, de Sousa LM, Pieczarka JC, Noronha RCR (2017) Karyotypic evolution and chromosomal organization of repetitive DNA sequences in species of *Panaque*, *Panaqolus*, and *Scobinancistrus* (Siluriformes and Loricariidae) from the Amazon Basin. *Zebrafish* 14: 251–260. <https://doi.org/10.1089/zeb.2016.1373>

Azpelicueta MM, Lundberg JG, Loureiro M (2008) *Pimelodus pintado* (Siluriformes: Pimelodidae), a new species of catfish from affluent rivers of Laguna Merín, Uruguay, South America. Proceedings of the Academy of Natural Sciences of Philadelphia 157: 149–162. [https://doi.org/10.1635/0097-3157\(2008\)157\[149:PPSPAN\]2.0.CO;2](https://doi.org/10.1635/0097-3157(2008)157[149:PPSPAN]2.0.CO;2)

Bertollo LAC, Takahashi CS, Filho OM (1978) Cytotaxonomic considerations on *Hoplias lacerdae* (Pisces, Erythrinidae). Revista Brasileira de Genética 2: 103–120.

Borin LA, Santos ICM (2002) Cytogenetic aspects in species of the genus *Pimelodus* (Pisces, Siluriformes, Pimelodidae) of the River Paraná Basin. Cytologia 67: 199–204. <https://doi.org/10.1508/cytologia.67.199>

Borin LA, Santos ICM (2004) Study on karyotype and occurrence of B chromosomes in two endemic species of the genus *Pimelodus* (Siluriformes, Pimelodidae) from the river Iguaçu. Hereditas 140: 201–209. <https://doi.org/10.1111/j.1601-5223.2004.01812.x>

Carvalho RA, Sanchez S, Swarça AC, Fenocchio AS, Santos ICM, Dias AL (2011) Chromosomal analyses in *Megalonema platanum* (Siluriformes: Pimelodidae), an endangered species from South American rivers. Neotropical Ichthyology 9(1): 177–182. <https://doi.org/10.1590/S1679-62252011005000008>

Della-Rosa VA, Bertollo LAC, Ferrari I, Takahashi CS, Moreira Filho O, Foresti F (1980) Estudos citogenéticos de peixes da Amazônia. II. Ordem Siluriformes. Ciência e Cultura 32: 735.

Dias AL, Foresti F (1993) Cytogenetic studies on fishes of the family Pimelodidae (Siluroidei). Revista Brasileira de Genética 16(3): 585–600.

Eigenmann CH (1912) The fresh-water fishes of British Guiana, including a study of the ecological grouping of species, and the relation of the fauna of the plateau to that of the lowlands. Memoirs of the Carnegie Museum 5(67): 1–578. <https://doi.org/10.5962/bhl.part.14515>

Eschmeyer WN, Fong JD (2017) Catalog of fishes. California Academy of Sciences. <https://www.calacademy.org/scientists/projects/catalog-of-fishes> [accessed 4 July 2018]

Farias AA, Brito JG, Venere PC (2000) Citogenética de Pimelodidae: Caracterização cromossômica de *Pimelodus blochii*, *Pimelodella cristata* e *Hemisorubim platyrhynchos* (Siluriformes) do médio Araguaia. Proceedings of VIII Simpósio de Citogenética e Genética de Peixes, Manaus, October 2000, Brazil.

Favarato RM, Silva M, Oliveira RR, Artoni RF, Feldberg E, Matoso DA (2016) Cytogenetic diversity and the evolutionary dynamics of rDNA genes and telomeric sequences in the *Ancistrus* genus (Loricariidae: Ancistrini). Zebrafish 13(2): 103–111. <https://doi.org/10.1089/zeb.2015.1140>

Fenocchio AS, Pastori MC, Lopez PA, Sanchez S, Alberdi AJ, Bordenave S, Dib MC (1994) Levantamento citogenético em peixes de água-doce da Argentina: resumo das espécies estudadas. Proceedings of V Simpósio de Citogenética Evolutiva Aplicada a Peixes Neotropicais, Botucatu, October 25–27, 1994, Brazil, 8.

Ferraris Jr CJ (2007) Checklist of catfishes, recent and fossil (Osteichthyes: Siluriformes), and catalogue of siluriform primary types. Zootaxa 1418: 1–628. <https://doi.org/10.11646/zootaxa.1418.1.1>

Ferreira M, Garcia C, Matoso DA, Jesus IS, Cioffi MB, Bertollo LAC, Zuanon J, Feldberg E (2017) The *Bunocephalus coracoideus* species complex (Siluriformes, Aspredinidae). Signs of a speciation process through chromosomal, genetic and ecological diversity. Frontiers in Genetics 8: 1–12. <https://doi.org/10.3389/fgene.2017.00120>

Ferreira M, Kavalco KF, Almeida-Toledo LF, Garcia C (2014) Cryptic diversity between two *Imparfinis* species (Siluriformes, Heptapteridae) by cytogenetic analysis and DNA barcoding. *Zebrafish* 11(4): 306–317. <https://doi.org/10.1089/zeb.2014.0981>

Garcia C, Filho OM (2008) Localization of ribosomal genes in three *Pimelodus* species (Siluriformes, Pimelodidae) of the São Francisco River: 5S genes as species markers and conservation of the 18S rDNA sites. *Genetics and Molecular Biology* 31(1): 261–264. <https://doi.org/10.1590/S1415-47572008000200018>

Girardi SC (2015) Citogenética Básica e Molecular em espécies de Pimelodidae (Siluriformes) coletadas nas Bacias do Rio Paraná e do Rio Uruguai: Uma Abordagem na Taxonomia e Sistemática. M.Sc. Dissertation, Cascavel, Brazil, State University of Western Paraná, 125 pp. [In Portuguese]

Howell WM, Black DA (1980) Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia* 36: 1014–1015. <https://doi.org/10.1007/BF01953855>

Levan A, Fredga K, Sandberg AA (1964) Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 107–110.

Lucinda PHF, Ribeiro FRV, Lucena CAS (2016) *Pimelodus quadratus*, a new long-whiskered catfish from the rio Tocantins drainage, Brazil (Siluriformes: Pimelodidae). *Ichthyological Exploration of Freshwaters* 27: 337–346.

Margarido VP, Gavasso E (2000) Análise cariotípica em *Pimelodus ortmanni* (Pisces, Siluriformes, Pimelodidae) coletado no Rio Iguaçu – Bacia do Iguaçu. Proceedings of 46º Congresso Nacional de Genética. Águas de Lindóia, September 19–23, 2000, Brazil, 63.

Martins C, Galetti-Jr PM (1999) Chromosomal localization of 5S rDNA Genes in *Leporinus* fish (Anostomidae, Characiformes). *Chromosome Research* 7: 363–367. <https://doi.org/10.1023/A:1009216030316>

Martins C, Vicari MR (2012) Hibridização in situ em cromossomos de peixes. In: Guerra M (Ed.) *Citogenética Molecular: Protocolos comentados*. Ribeirão Preto, 59–87.

Martins C, Wasko A (2004) Organization and evolution of 5S ribosomal DNA in the Fish genome. In: Williams CR (Ed.) *Focus on Genome Research*. New York, 335–363.

Mazzuchelli J, Swarça AC, Dias AL (2007) Structural chromosome polymorphism in a *Pimelodus maculatus* La Cepéde, 1803 population (Siluriformes, Pimelodidae) from the Paranapanema River Basin, PR, Brazil. *Brazilian Journal of Biology* 67(4): 935–937. <https://doi.org/10.1590/S1519-69842007000500018>

Neto AM, Silva M, Matoso DA, Vicari MR, Almeida MC, Colares-Pereira MJ, Artoni RF (2011) Karyotype variability in Neotropical catfishes of the family Pimelodidae (Teleostei: Siluriformes). *Neotropical Ichthyology* 9(1): 97–105. <https://doi.org/10.1590/S1679-62252011005000002>

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. *Proceedings of the National Academy of Sciences of the United States of America* 83(9): 2934–2938. <https://doi.org/10.1073/pnas.83.9.2934>

Ramirez-Gil H, Feldberg E, Almeida-Val VMF, Val AL (1998) Karyological, biochemical and physiological aspects of *Callophysus macropterus* (Siluriformes, Pimelodidae) from the Solimões and Negro rivers (Central Amazon). *Brazilian Journal of Medical and Biological Research* 31: 1449–1458. <https://doi.org/10.1590/S0100-879X1998001100014>

Ribeiro FRV, Lucena CAS (2006) Nova espécie de *Pimelodus* (Siluriformes, Pimelodidae) dos rios Tapajós e Tocantins, Brasil. *Iheringia Serie Zoologia* 96(3): 321–327. <https://doi.org/10.1590/S0073-47212006000300008>

Rocha MS (2006) Sistemática e Filogeografia de *Pimelodus blochii* (Siluriformes: Pimelodidae) da Amazônia. M.Sc. Dissertation, Manaus, Brazil: National Institute of Research on Amazonia, Federal University of Amazonas, 82 pp. [In Portuguese]

Rocha RH, Baumgärtner L, Paiz LM, Margarido VP, Fernandes CA, Gubiani EA (2016) An uncommon co-localization of rDNA 5S with major rDNA clusters in Callichthyidae (Siluriformes): a report case in *Corydoras carlcae* Nijssen & Isbrücker, 1983. *Comparative Cytogenetics* 10(4): 603–613. <https://doi.org/10.3897/compcytogen.v10i4.9507>

Sánchez S, Fenocchio AS, Jorge LC (2000) Estudios citogenéticos en peces de la familia Pimelodidae (Pisces, Siluriformes) de la cuenca del río Paraná, Argentina. Análisis cromosómico de dos especies de Luciopimelodinae. *Comunicaciones Científicas y Tecnológicas*. Universidad Nacional del Nordeste. http://www.unne.edu.ar/unnevieja/Web/cyt/cyt/2000/4_veterinarias/v_pdf/v_050.pdf [Accessed 21 November 2017]

Silva LVB, Abreu MF, Souza IL, Venere PC (2004) Caracterização Cromossômica de *Hemisorubim platyrhynchos*, *Sorubim lima* e *Pimelodus blochii* (Pisces, Siluriformes, Pimelodidae) do Médio Araguaia. *Proceedings of X Simpósio de Citogenética e Genética de Peixes*. Natal, October 2004, Brazil, 132.

Souza L, Swarça AC, Dias AL (2004) Analysis of the nucleolus organizer regions in 5 species of the genus *Pimelodus* (Siluriformes, Pimelodidae), using AgNO₃, CMA3 and FISH with the 18S rDNA probe. *Caryologia* 57(2): 144–150. <https://doi.org/10.1080/00087114.2004.10589384>

Souza L, Giuliano-Caetano L, Dias AL (2003) Karyotypic Study of three species of *Pimelodus* (Pisces, Pimelodidae) from the Paraguai River Basin. *Cytologia* 68(4): 345–350. <https://doi.org/10.1508/cytologia.68.345>

Summer AT (1972) A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research* 74: 304–306. [https://doi.org/10.1016/0014-4827\(72\)90558-7](https://doi.org/10.1016/0014-4827(72)90558-7)

Supiwong W, Liehr T, Cioffi MB, Chaveerach A, Kosyakova N, Fan X, Tanee T, Tanomtong A (2014) Comparative cytogenetic mapping of rRNA genes among naked catfishes: implications for genomic evolution in the Bagridae family. *Genetics and Molecular Research* 13(4): 9533–9542. <https://doi.org/10.4238/2014.November.12.2>

Swarça AC, Fenocchio AF, Dias AL (2007) An update Cytogenetic Review for Species of the Families Pseudopimelodidae, Pimelodidae and Heptapteridae (Pisces, Siluriformes). Suggestion of a Cytotaxonomical Classification. *Caryologia* 60(4): 338–348. <https://doi.org/10.1080/00087114.2007.10797957>

Terencio ML, Almeida MC, Artoni RF (2001) Citogenética de *Pimelodus ortmanni*, uma espécie de mandí endêmica ao rio Iguaçu. *Proceedings of 47º Congresso Nacional de Genética*. Águas de Lindóia, October 2–5, 2001, Brazil, 135.

Treco FR, Dias AL (2009) Karyotypes of Two Species of the Genus *Pimelodus* (Siluriformes, Pimelodidae). *Folia Biologica (Kraków)* 57(1/2): 43–48.

Vasconcelos C, Santos ICM (2000) Chromosome polymorphism in species of the Pimelodidae (Teleostei, Siluriformes). *Hereditas* 132: 103–109. <https://doi.org/10.1111/j.1601-5223.2000.00103.x>